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Differential Effects of Short- and Long-Pulsewidth Laser Exposures on Retinal Ganglion Cell Response

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Time-dependent effects of laser exposures on rhesus monkey retinal ganglion cells were studied with a Q-switched, doubled Nd:glass laser, which produced 20 nsec pulses of 530-nm light, and a continuous-wave (CW) argon laser (514.5 nm), which produced exposures of 0.1-msec to 0.1-sec duration. Ganglion cell activity was recorded in situ by means of an intraocular electrode. Ganglion cells exposed to a single 20-nsec exposure, at a sublesion intensity, produced a 60–90 sec discharge of action potentials and exhibited a 2 log or greater elevation of light threshold, depending on beam size and intensity. At equivalent energy levels, the longer exposures produced the same or slightly weaker effects. This result is not as straightforward as it seems. Submillisecond flashes bleach no more than 50% of the visual pigment because of photoregeneration. The Dowling-Rushton relation predicts that a 50% bleach should produce only a 1.5 log loss of cone sensitivity. Exposures longer than 1 msec should not photoregenerate pigment (ie, more pigment will be bleached for a given exposure intensity). In view of the probable differences in pigment bleaching, it appears that the Q-switched laser light adapts the cells out of proportion to the visual pigment actually bleached—a single-cell analogue of Rushton's "theta" effect.

Key words: Q-switched lasers; neodymium lasers; argon lasers; reciprocity, electrophysiology

INTRODUCTION

Q-switched lasers are being utilized in several clinical applications, particularly in ophthalmic surgery. Although the high-power effects of Q-switched laser exposures have been discussed at length in the literature, the effects of sublesion exposures have not received much attention. The short pulses these lasers are capable of emitting have subtle bioeffects that may interest ophthalmologists and other practitioners using the neodymium:YAG (Nd:YAG) or Nd:glass laser for surgical purposes. This article is the result of investigations of retinal function following low-level exposures from an Nd:glass laser, using *in vivo* recordings of ganglion cell activity from the rhesus monkey retina.

About 30 years ago, Hagins demonstrated that a short (100 μsec or less) flash of light bleaches no more than about 50% of the visual pigment, regardless of the intensity of the flash [Hagins, 1956]. This observation has been confirmed many times since, for both cone [Rushton and Baker, 1963] and rod pigment [Pugh, 1975]. This phenomenon, termed photoregeneration, is thought to be due to a susceptible intermediate of the visual pigment bleaching cycle absorbing a second photon within 1 msec after the first photon hit (Fig. 1). The second photon absorption causes the molecule to be returned to the unbleached state, either to rhodopsin or to an isopigment, (eg, isorhodopsin). It has been proposed that pigment molecules receiving an even number of photon hits will be photoregenerated, whereas an odd number of hits will result in the molecule completing the visual bleaching cycle [Williams, 1964]. An intense light flash delivering all its photons within the critical period causes the visual pigment molecules to undergo multiple photon hits and so is an effective photoregenerator of pigment.

Rushton and Baker [1963] were the first to study the visual consequences of photoregenerating flashes. They found that the effect on visual sensitivity of a 1-msec flash was similar to that of a long (10 sec) bleaching light delivering the same number of quanta to the observer's eye. The 10-sec light bleached over 99% of the

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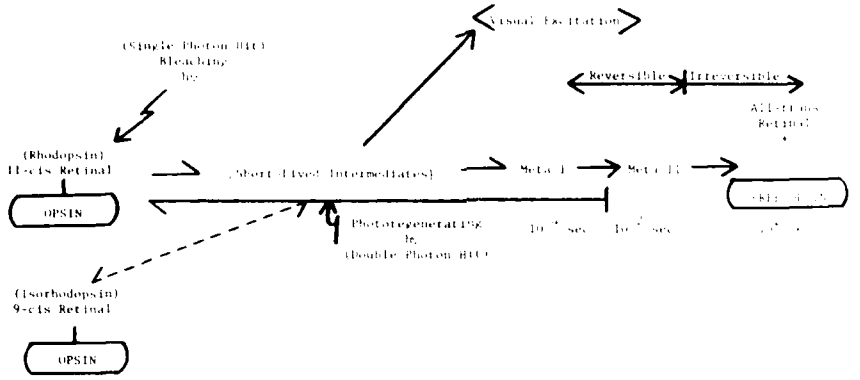

Fig. 1. Photoregeneration of visual pigment. The usual bleaching process involves a molecule of rhodopsin absorbing a single photon and proceeding through a series of short-lived intermediates until the metarhodopsin II state is reached. Meta II is irreversible, and the forward reaction continues until the chromophore completely dissociates from the opsin protein. If a second photon is absorbed before the meta II stage, the reaction can be reversed, and an "unbleached" pigment molecule is restored. Not all the regenerated pigment may be rhodopsin (11-cis retinal + opsin); some fraction may exist as an isopigment such as isorhodopsin (9-cis retinal + opsin). This schematic illustrates the bleaching/regeneration pathway for rhodopsin, but regeneration of the cone pigments probably follows a similar path.

visual pigment, whereas the 10-μsec flash bleached only about 50%. Their finding appeared to violate one of visual science's empirical "laws," ie, the Dowling-Rushton relation [Rushton, 1961; Dowling, 1963], which held that the log of the visual threshold is proportional to the amount of bleached pigment in the photoreceptors. This paradoxical result was termed the "theta" effect [Rushton and Baker, 1963] and has been reproduced under a variety of conditions [Hollins and Alpern, 1973; Pugh, 1975].

The 20-nsec pulse width produced by the Q-switched laser of the present study is clearly in the photoregenerating time domain. The purpose of these experiments was to compare the retinal response to short- and long-pulse-width laser exposures. When equated for exposure intensity, 20-nsec exposures produced by the doubled output (530 nm) of the Nd:glass laser were just as effective in exciting ganglion cells as were longer exposures derived from the continuous-wave (CW) argon laser.

MATERIALS AND METHODS

Experimental Animals and Surgical Procedures

Recordings of ganglion cell activity were obtained from ten adult, male rhesus monkeys (*Macaca mulatta*) weighing between 4.0 and 7.0 kg. The recordings were made over the course of three to four sessions per animal; all animals were recovered following the experimental sessions.

The ganglion cell activity was recorded in the retina with an intraocular electrode. Access into the eye for the microelectrode was obtained through a 2–3 mm incision parallel to the limbus through the pars plana. Animals were tranquilized with ketamine HCl (15 mg/kg, IM). A surgical plane of anesthesia was achieved by an initial injection of Nembutal (20–30 mg/kg, IV) followed by a maintenance infusion of 1.5–3 mg/kg/hr. After the animal was secured in a stereotaxic holder, it was paralyzed with an infusion of gallamine triethiodide at a rate of 2 mg/kg/hr and artificially respirated. The pCO_2 in the expired air was maintained at 3.5–5%. The animal's temperature, EKG, EEG, and pCO_2 were monitored throughout the session and maintained within physiological limits for the monkey.

A cannula that fit tightly into the pars plana incision was inserted and held in place with a specially designed clamp [Wormington and Jaeger, 1983]. This cannula had a reservoir attached to it that could be raised to maintain the intraocular pressure at approximately 18 mmHg. A clear contact lens was placed over the animal's eye to prevent corneal desiccation. The stereotaxic holder with the animal was then placed at position "ME" of the optical train (Fig. 2), which allowed the laser as well as incoherent light to be directed into the animal's eye.

At the end of the experiment (typically after 10–12 hr), the cannula was removed and the incision closed with 7-0 absorbable suture. The wound area was covered with a chloramphenicol (chloromycetin) ophthalmic ointment, and the animal was given a systemic dose of chloramphenicol (75 mg/kg, IV).

Optical and Laser Systems

A schematic diagram of the optical system is shown in Figure 2 (the letters in parentheses in the following description refer to components labeled in Figure 2). The monkey viewed a light stimulus, an 18° (visual angle) background field, and the laser through a three-channel Maxwellian view system. A xenon arc lamp (X) was the source of the test stimulus, formed by the lenses L2–L4. The test stimulus was a spot of light that was directed onto the ganglion cell's receptive field. This light spot

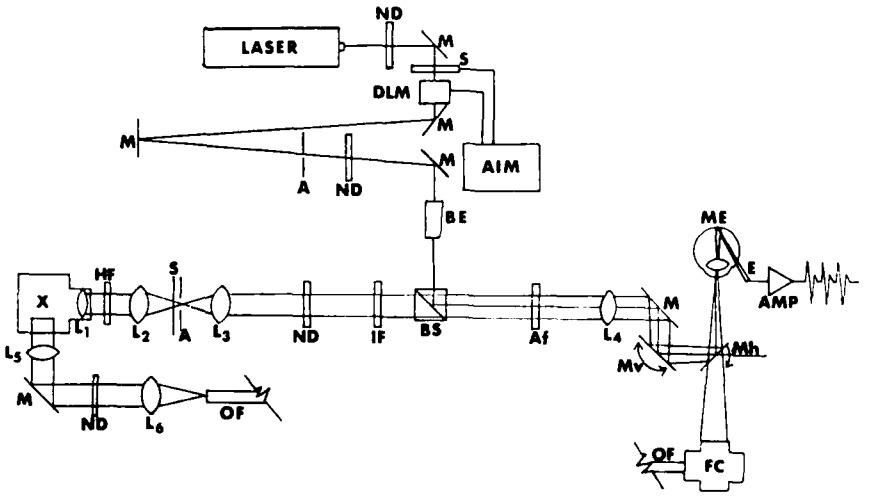

Fig. 2. System used to deliver coaxial incoherent and laser exposures to retinal ganglion cells in vivo. Note: This apparatus constituted a three-channel, Maxwellian view stimulator, two channels for incoherent light and one for the laser. A, apertures; Af, Maxwellian field aperture; AIM, Rockwell AIM microcomputer; AMP, Grass P16 amplifier; BE, laser beam expander; BS, prism beam splitter; DLM, acoustic-optical modulator; E, microelectrode; FC, fundus camera; HF, heat-absorbing glass; IF, interference filters; LASER, Spectra Physics 171 argon or Apollo Model 5 Nd:glass; L1,3,5, collimating lenses; L4, Maxwellian field lens; L2,6, condensing lenses; M, fixed front surface mirrors; ME, monkey (eye); Mv,Mh, movable mirrors for vertical and horizontal beam deflection; ND, neutral density filters; OF, optic fiber for adapt. field; S, electromechanical shutters; X, 150-W xenon arc lamp.

typically was 400 msec in duration and was presented every 1.5 sec. The intensity and wavelength were varied according to the response of the cell. The test stimulus was used to determine the receptive field extent of the ganglion cell as well as its light response (ON or OFF center, color opponent or nonopponent).

A second channel was derived from the xenon arc lamp and was conducted into the flash port of the fundus camera through a fiber optic cable (OF). This channel produced an 18° field on the monkey's retina, which was used for setting the adaptation level. The wavelength and intensity of this channel could be adjusted separately from the main channel.

Two lasers were used in the course of this study. The Q-switched laser source was an Apollo Model 5 Nd:glass laser, which produced 20-nsec pulses of 1,060-nm light. This beam was passed through a potassium dihydrogen phosphate (KDP) crystal to obtain frequency-doubled (530 nm) radiation. Any 1,060-nm wavelength light leaving the KDP crystal was blocked by a pair of KG-3 filters. The 530-nm light was joined with the main xenon beam at the beam splitter (BS) so that the two sources were collinear. Both the xenon and the laser beams were apertured by the field stop, Af, to produce retinal spot sizes of 0.125°, 0.25°, 0.5°, or 2.0° of visual angle. The combined beams were then focussed on the pupil plane by the Maxwellian field lens L4. A pair of orthogonal, movable mirrors (Mh and Mv) mounted between L4 and the monkey's eye positioned the resulting image to the required position on the retina.

Laser exposures with pulse widths between 0.1 and 0.0001 sec were obtained with the 514.5-nm line from a CW argon gas laser (Spectra Physics 171). Various pulse widths were obtained by passing the laser output through a mechanical shutter (S2) and a DataLight DLM-1 acoustooptical modulator (DLM). A microcomputer (AIM) was programmed to open and close the mechanical shutter prior to and after the DLM was activated for the required pulse width. The mechanical shutter served to block any laser light leaking through the DLM. The DLM was activated by a control signal from the AIM to provide pulse widths of 0.0001, 0.001, 0.01, and 0.1 sec. The beam emerging from the DLM was expanded with a beam telescope (BE) and joined with the channel from the xenon lamp at beam splitter (BS). The rest of the optical path was identical to that described for the Apollo laser.

The output of the laser was measured at two locations: at the BS, where the laser joined the light from the xenon, and at point ME, where the monkey's eye was normally positioned. These two measurements established a ratio between energy actually arriving at the eye and that passing through the BS. This ratio was used to scale energy measurements made at the BS during experiments. The output of the Apollo laser was measured with a Laser Precision Rk3230 pulse detector as well as with an EG&G 460 laser power meter. The argon laser was monitored with the EG&G meter. The maximum energy per pulse obtained with the Apollo laser, incident at the cornea, was 6.5 μJ for a 2° retinal image size. An

exposure of this magnitude would deliver 65% of the maximum permissible exposure (MPE) for an extended source according to the ANSI Z136-1 safety standard. The most intense exposure used in the study was 63% of the MPE, which delivered a retinal illuminance (at 530 nm) of 6.78 log troland-seconds (td-s) [Glickman, 1987].

Recording Methods

Single-neuron recordings were made with tungsten-in-glass microelectrodes similar to those described by Levick [1972]. The electrodes were passed through the cannula into the eye. Inside the cannula was a compressible O-ring, which made a water-tight seal with the electrode shank [Wormington and Jaeger, 1983]. The water-tight system served to maintain the intraocular pressure. The optical system (Fig. 2) included a fundus camera, which allowed the experimenter to observe the interior of the eye while manually guiding the microelectrode back to the desired point on the retina. The electrode was advanced until it just touched the retinal surface (which could be detected visually by a slight "darkening" around the electrode tip). The electrode position was then adjusted until ganglion cell spike activity was recorded. Spike data were displayed on an oscilloscope and stored on magnetic tape for later computer analysis. The computer made spike counts for time epochs ("sweeps") consisting of 200 msec prior to the stimulus, the 400-msec stimulus, and 800 msec following the stimulus.

Experimental Procedures

The response of ganglion cells to equal-energy exposures of different pulse widths was measured as the number of spikes produced by the laser exposure. Prior to the laser exposure, each cell was brought to within 1 log unit of the same (photopic) adaptational state. The test stimuli were presented against a dark background (ie, backgrounds were not used). A baseline measure, including both spontaneous activity and spikes evoked by the test stimulus, was obtained for each cell for the ten sweeps preceding the presentation of a laser flash. At the end of this baseline period, the laser flash was presented and a count of the impulses was made for all sweeps following the laser until the spikes per sweep returned to within 10% of the baseline level. This cumulative count constituted the laser response. A ganglion cell was not subjected to a second laser exposure until its firing rate returned to the original baseline level.

Since the pulse width of the Nd:glass laser could not be varied, the responses to the Q-switched laser were obtained in separate experiments in which only the exposure intensity was varied (from 63% down to 1% of the MPE, ie, down to 1.1×10^{-7} J). The argon laser was used in subsequent experiments in which the pulse width and intensity were adjusted as previously described so as to produce a series of quantum-equivalent exposures. The optical arrangement used to chop the CW output of the argon laser, however, also limited the intensity of the exposures that could practicably be achieved. The argon laser exposures ranged from 0.0001 to 0.1 sec, and the total energy delivered to the eye ranged from 7×10^{-13} to 1.3×10^{-7} J. Thus the only overlaps between the argon and Nd exposures were for those delivering approximately 10^{-7} J to the eye.

RESULTS

Effect of Q-Switched Laser Exposures

ON-center ganglion cells could be driven into a prolonged afterdischarge of action potentials when exposed to a single 20-nsec flash. To obtain this type of response, the beam intensity had to be > 10% of the MPE, and the beam diameter at least 0.25°. For exposures delivering > 50% of the MPE, the afterdischarge lasted for 50–145 sec (Fig. 3, filled circles). During this time, the light sensitivity of the cell was depressed. Following an exposure with the 0.5° or 2.0° beam, the light threshold of the cells was initially raised by at least 1.5 log units (Fig. 3, filled squares). The actual increase could only be estimated; immediately after the laser exposure these cells did not respond to the brightest test stimulus available (4.23 log td). It was demonstrated, however, that six of 14 cells had initial threshold increases of > 2.5 log units. For 50% MPE or greater laser flashes, the cell required up to 10 sec for the threshold to fall back into the range of the stimulator, and another 20–175 sec to reach the prelaser threshold, depending on the initial sensitivity of the cell. Table I shows the mean afterdischarge durations and light threshold recovery times for laser exposures made with 0.25°, 0.5°, and 2.0° beams.

CW Laser Exposures

These experiments were designed to test the hypothesis that quantum-equivalent exposures elicit retinal responses of similar magnitude, regardless of pulse-width. Four retinal ganglion cells were subjected to quan-

TABLE I. Response of On-Center Ganglion Cells to a 60% MPE Exposure From an Nd:Glass Laser

Laser beam diameter (°)	No. of cells	Duration of afterdischarge (s)[a]	Time to recovery of light threshold	Average initial sensitivity loss (log)[a]
2.0	3	83.3 ± 19.6	60.0 ± 13.0	2.17 ± 0.14
0.5	8	64.0 ± 12.0	59.6 ± 7.6	2.43 ± 0.26
0.25	8	28.0 ± 12.0	16.8 ± 7.1	1.58 ± 0.18
0.125	3	Negligible	Negligible	Negligible

[a] ± SEM.

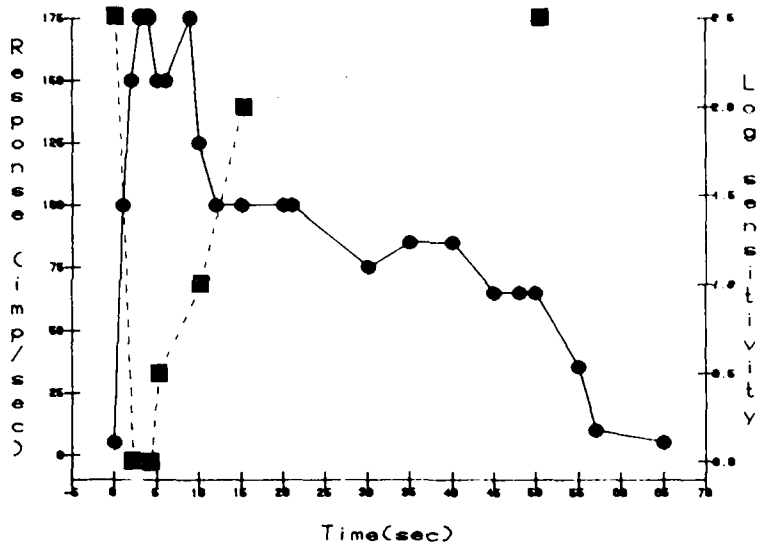

Fig. 3. Ganglion cell response to a single 20-nsec laser exposure at 60% MPE. The laser was presented at time 0. Laser beam diameter was 0.5°. Filled squares; sensitivity of cell, tracked by changing the attenuation of the test stimulus (right ordinate). Increasing values on this scale indicate recovery of the cell. Filled circles, firing frequency of cell (left ordinate). Note the oscillations in the afterdischarge frequency during the first 15 sec following the laser.

tum-equivalent laser exposures delivered over different durations (0.0001–0.1 sec). Exposures were made at several energy levels. At the low end, the exposures were just above threshold for the cell; at the upper end, they elicited prolonged trains of action potentials from the cell.

For three of the cells, reciprocity between intensity and exposure duration was found for all durations and intensities used. The results from such an experiment are shown in Figure 4. For a given exposure intensity, the response of the cell was similar. This can be seen in Figure 4 as clustering of the points at a given exposure energy. The cell's response showed a monotonic increase with increasing exposure energy.

One cell, when tested with the 0.125° beam diameter, did not show this monotonic increase. The response failed to increase when the input energy was increased above 10^{-11} J. This same cell, however, did show reciprocity when tested with larger beam diameters. The failure of the cell to respond to the small diameter beam may, therefore, have been due to an incorrect alignment of the beam in the cell's receptive field.

When the data from the CW and Q-switched laser exposures were combined, the trend of increasing cellu-

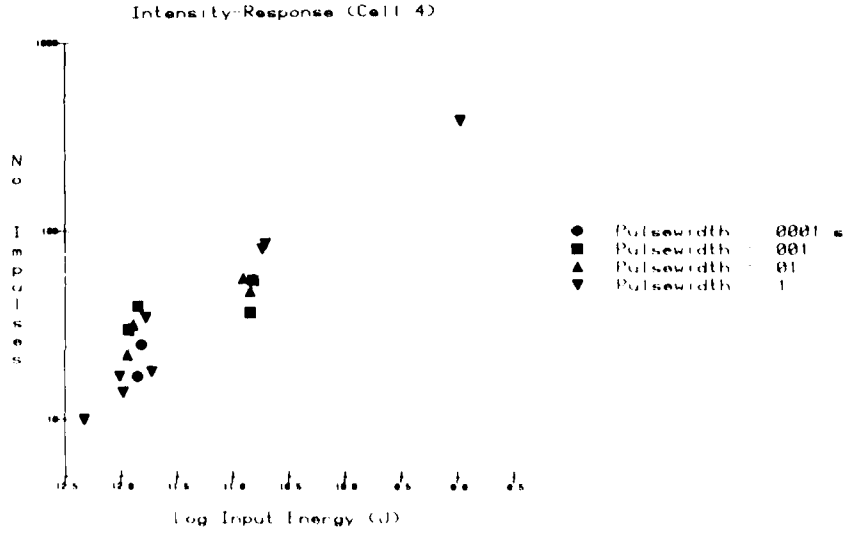

Fig. 4. Intensity-response relation of a retinal ganglion cell. The responses of the cell are recorded as the number of impulses produced by each laser exposure. The cell gives a similar response to laser exposures delivering the same total energy to the eye regardless of the pulse width. Laser beam diameter was .125°. Pulse widths as indicated.

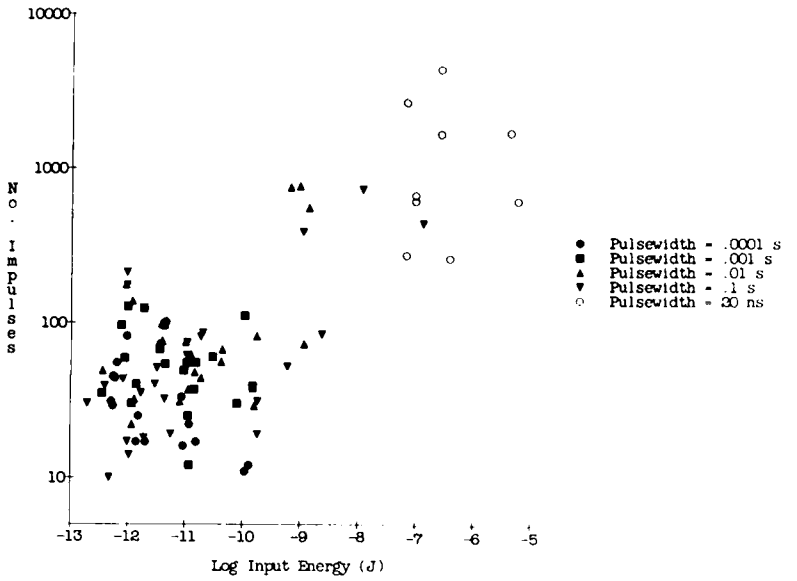

Fig. 5. Intensity-response relation of retinal ganglion cells to laser exposures, with pulse widths ranging from 0.1 to 2×10^{-8} sec. This experiment is similar to that in Figure 4. Data from 13 ganglion cells are shown. Filled symbols, data from four ganglion cells exposed to the argon laser, pulse widths from 0.1 to 0.0001 sec. Open circles, data from nine ganglion cells exposed to 20-nsec pulses from the Nd:glass laser.

lar response with higher exposure energy was apparent (Fig. 5). In Figure 5, the pulse width of each exposure is distinguished by the plotting symbol. As before, the cell response was measured as the number of impulses evoked by the laser. Although there is inherent noise in nonnormalized, single-neuron data of this sort, it appears that the monotonic relationship, just noted, between the cell's response and the laser energy is maintained. At a first approximation, then, reciprocity holds over the range of exposure durations used in this experiment.

DISCUSSION

The interpretation of these results requires several qualifications. First, because of differences in the response properties of different neurons, substantial scatter in the data must be expected. All the neurons in this study were recorded from the central 6° of retina. In this way, we hoped to minimize scatter by recording from a relatively homogeneous population of neurons. Second, equipment needed to measure directly the amount of bleaching actually produced by the various laser exposures was not available in this laboratory. Therefore, the photoregeneration of pigment by exposures briefer than 1 msec must be surmised from the work of others. For example, Catt et al [1983] reported regeneration of pigments following a 3 μsec flash from a dye laser. They also found that the degree of photoregeneration depended on the wavelength of the bleaching light. It is important to consider the process of pigment photoregeneration because it could affect the time course of the later, pigment-mediated phase of dark adaptation following a brief, intense flash. While the rapid recovery of light sensitivity that occurred in the first 10 sec following the laser exposure probably reflects neural adaptation processes, the slower phase after 10-15 sec is due to photochemical regeneration of visual pigment (see Fig. 3). This biphasic recovery is characteristic of the cone-dominated portion of dark adaptation [Crocker et al., 1980].

If the assumption is accepted that photoregeneration was produced by the shortest pulse widths used in this study, then the conclusion must follow that reciprocity between intensity and duration occurs, with respect to retinal response, despite a difference in pigment bleaching. An estimate of this difference can be made. According to Rushton [1972], a nonphotoregenerating bleaching light delivering 6.78 log td-s (the illuminance of the 60% MPE 530-nm laser exposure) will bleach 72% of the cone pigment. By the Dowling-Rushton relation, this level of bleach should result in a ~2.3 log increase in threshold. The photoregenerating 20-nsec flash, however, maximally bleaches ~50% of the pigment, which should result in only a 1.5 log unit increase in threshold. Most of the ganglion cells exposed to the 60% MPE Nd:glass laser had initial threshold increases $>$ 1.5 log units—$>$2.5 log units in six cases. Thus the single-neuron response resembles the psychophysical observations cited in the Introduction. Similarly, Ernst and Kemp

[1979] reported that the ERG amplitude recorded from the frog eye is the same after photoregenerating and nonphotoregenerating flashes.

The mechanism for the "paradoxical" effect of photoregenerating flashes has not yet been established. Various theories have been advanced. Regenerated isopigment has been supposed to screen normal pigment from incoming photons [Rushton and Baker, 1963]. It is known that the interconversion of isorhodopsin to metarhodopsin in vitro has a quantal efficiency about 30% that of the rhodopsin to metarhodopsin reaction [Kropf and Hubbard, 1958]. Thus it is possible that the isopigment does participate in phototransduction but at a low level of efficiency. More recently, it has been observed that the event that reduces sensitivity after a photoregenerating flash occurs soon after photon absorption, before metarhodopsin is produced [Pugh, 1975]. This time scale would correspond to the release of the photoreceptor internal transmitter. The magnitude of the photoreceptor response, and that of all postsynaptic retinal neurons, would then depend on the amount of internal transmitter released, and, hence, on the size of the initial photon catch, rather than on the fraction of visual pigment ultimately bleached by a given light exposure.

CONCLUSIONS

This study demonstrates that the light-evoked retinal response rises monotonically with the intensity of a nondamaging laser exposure regardless of pulse width. The reciprocity between intensity and exposure duration extends down to the 20-nsec pulse width produced by the O-switched Nd laser. Submillisecond exposures, however, bleach less visual pigment than do longer, quantum-equivalent exposures because of photoregeneration. Therefore, the amplitude of the visual response is proportional to the number of photons delivered by the light, not to the amount of visual pigment actually bleached.

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